Original Article Clinics in Orthopedic Surgery 2025;17:138-147 • https://doi.org/10.4055/cios24125

Changes in Gene Expression of the Extracellular Matrix in Patients with Full-Thickness Rotator Cuff Tears of Varying Sizes

Jian Jiang, MD*,†, Kwi-Hoon Jang, PhD†, Sung Yong Ahn, PhD*,†, Chris Hyunchul Jo, MD*,†

*Department of Translational Medicine, Seoul National University College of Medicine, Seoul,
†Department of Orthopedic Surgery, SMG-SNU Boramae Medical Center, Seoul National University College of Medicine, Seoul, Korea

Background: This study aimed to investigate changes in gene expression related to matrix synthesis in individuals with full-thickness rotator cuff tears (RCTs) and normal tendon tissues. The study also aimed to examine the differences in gene expression according to 4 distinct tear sizes.

Methods: A total of 12 patients with full-thickness RCTs were included in the study, all of whom underwent arthroscopic rotator cuff repair. The RCTs were stratified by size into small, medium, large, and massive. Tendon samples were harvested from the midpoint between the lateral end of the torn tendon and the musculotendinous junction. Subsequent analysis of the tissue samples revealed the mRNA expression levels of 11 collagen types, 6 proteoglycans, and 8 glycoproteins through real-time polymerase chain reaction techniques. For control purposes, supraspinatus tendon tissue was sourced from 3 patients who had proximal humerus fractures but did not present with RCTs.

Results: Among the 11 collagens and 14 non-collagenous protein (NCP) genes examined in this study, *COL3A1* and *COL10A1* showed a significant increase, whereas *COL4A1* and *COL14A1* showed a tendency to decrease compared to those in the normal group. *ACAN* significantly increased by 8.92-fold ($p < 0.001$) compared to that in the normal group, whereas *DCN* and *LUM* showed a tendency to decrease. *FN1* and *TNC* increased significantly by 3.47-fold ($p = 0.003$) and 5.38-fold ($p = 0.005$), respectively, and the genes *ELN*, *LAMA2*, and *THBS1* were all significantly reduced compared to those in the normal group. In the NCPs, almost all the genes with increased expression levels had the highest level in small size RCTs, and gene expression decreased as the size increased. The 3 proteoglycans (*ACAN*, *BGN*, and *FMOD*) showed the highest levels of expression in small size RCTs compared to those in the normal group, and 5 glycoproteins (*COMP, FBN1, FN1, HAPLN1*, and *TNC*) also showed the highest expression in small size RCTs.

Conclusions: We confirmed that most of the detected extracellular matrix gene expression changes were related to the size of the full-thickness RCTs. In NCPs, gene expression was increased in small-size tears, and gene expression levels were significantly reduced when the size increased.

Keywords: *Rotator cuff tear, Extracellular matrix, Non-collagenous protein, Gene expression, Tear size*

The occurrence of rotator cuff tears (RCTs), which is a common cause of shoulder pain, often brings a low quality of life and a heavy financial burden to patients.[1] The timing of a surgical intervention is critical, and studies have shown that a prompt surgical intervention for RCTs can often lead to better outcomes.[2] Nonetheless, for patients with full-thickness tears, the problem of postoperative re-tears (rate up to 94%) remains unresolved.[3]

Received May 22, 2024; Revised May 23, 2024;
Accepted May 28, 2024
Correspondence to: Chris Hyunchul Jo, MD
Department of Translational Medicine, Seoul National University College of Medicine, 103 Daehak-ro, Jongno-gu, Seoul 03080, Korea
Tel: +82-2-870-2315, Fax: +82-2-870-3864
E-mail: chrisjo@snu.ac.kr

The full-thickness RCTs can be classified into 4 types based on the size of the tear[4]: small, medium, large, and massive. As the size of the tear increases, a decrease in activity of fibroblasts and angiogenesis leads to a loss in the repair ability of tendon tissue,[5] which not only affects the choice of a therapeutic or surgical method and the recovery process, but also influences the recurrence rate of tear. Studies have shown that patients with asymptomatic RCTs or small tears are at risk of experiencing an enlargement of the tear and a decline in muscle quality.[6] In contrast, the repair of small and medium size tears has offered promising outcomes even in elderly patients.[7]

Tendon degeneration is an active cell-mediated process involving synthesis, degradation, and remodeling of the extracellular matrix (ECM), considered a result of an imbalance in the synthesis and degradation of ECM.[8,9] The ECM, a complex meshwork of proteins, is a fundamental component of multicellular organisms, and it contains a large proportion of collagen with non-collagenous constituents such as proteoglycans and glycoproteins.[10] Collagen fibrils are the principle tensile element in a tendon and provide mechanical stability to the tendon and regulate cell adhesion and differentiation,[11] and non-collagenous ECM also acts as a cell-resident scaffold, regulating cues for cell behavior.[12]

Therefore, knowledge about the ECM in torn rotator cuff tissue would be crucial for elucidating the molecular mechanisms of the cause and progression of the tendon degeneration and may provide some clues for clinical treatment. Several studies compared differences in the expression of typical ECM genes in patients with RCTs, but did not specifically focus on the ECM and lacked related information[13,14] either the size of the tear was not compared or the classification was not comprehensive.[15,16] This study aimed to focus on investigating alterations in gene expression related to matrix synthesis in full-thickness RCTs and normal tendon tissues. Since up to 85% of the dry weight in a tendon is collagen, we divided the tendon ECM into 2 types, collagen and non-collagenous proteins (NCPs), to compare the mRNA expression levels of 11 of the major collagens and 14 NCPs in the tendon and analyze the differences among 4 different tear sizes.[17] We examined variations in the expression of 25 ECM genes among patients with and without full-thickness RCT to propose a correlation between the expression of ECM genes and the size of tears to offer valuable pointers for clinical interventions.

METHODS

This study was approved by the Institutional Review Board of SMG-SNU Boramae Medical Center (IRB No. 06-2012-78), and all patients provided written informed consent.

Study Design and Patients

From April 2008 to October 2008, patients were enrolled in this study according to the following inclusion and exclusion criteria. In the experimental groups, the inclusion criteria were full-thickness RCTs with delamination and available tissue samples of the rotator cuff tendon harvested at the time of surgery. The exclusion criteria were partial-thickness RCTs, inflammatory arthritis, including rheumatoid arthritis, a history of acute trauma and infection, previous subacromial injection within the past 3 months, systemic conditions associated with chronic pain, isolated subscapularis tear, rotator cuff arthropathy, calcific tendinitis, retear, a coagulation abnormality, a hepatic disease, or drug abuse and absence of tissue samples. Patients who had proximal humeral fractures but did not present with RCTs were included in the control group.

Surgical Procedures and Tissue Harvest

All surgical procedures were performed by a single surgeon (CHJ) by arthroscopic surgery. All arthroscopic surgeries were performed with patients in the lateral decubitus position under general anesthesia as previously described.[18] Briefly, systemic glenohumeral joint and subacromial exploration were performed, and lesions were managed as needed. Full-thickness supraspinatus (SST) samples were harvested en bloc in the middle portion of the tendon (between the lateral end of the torn tendon and the musculotendinous junction) using a biopsy punch with a diameter of 3 mm. The size of an RCT was classified according to DeOrio and Cofield[4] based on the greatest dimension as either small (< 1 cm), medium (1–3 cm), large (3–5 cm), or massive (> 5 cm). Tendon samples were harvested during an arthroscopic rotator cuff repair using a biopsy punch with a diameter of 3 mm. After gentle debridement of overlying bursa avoiding tissue contamination, samples from the margin of the RCTs were obtained and stored at –80 °C until use.

Real-Time Reverse Transcriptase Polymerase Chain Reaction for 3 Types of ECM

Total RNA was extracted, and reverse transcription and amplification were performed as previously described.[19] Briefly, total RNA was extracted using a Qiagen RNeasy mini kit (Qiagen) and quantified using a Nano Drop ND-

100 spectrophotometer (NanoDrop). First-strand complementary DNA (cDNA) was synthesized using the Superscript III Reverse Transcription kit (Invitrogen). Briefly, first-strand cDNA was synthesized from cellular mRNAs (1 μg) by heating a mixture (1 μg mRNA, 1 μL Oli-go(dT) 20 [50 uM], 1μL dNTP [10 mM], and up to 10 μL DW) to 65 °C for 5 minutes, cooling on ice for 1 minute, and then adding a mixture containing 2μL 10× RT buffer, 4 μL $MgCl_2$ (25 mM), 2 μL dithiothreitol (0.1 M), 1 μL RNaseOut (40 U/mL), and 1μL Superscript III Reverse Transcriptase (200 U/mL) (Invitrogen). The reaction mixture was held at 50 °C for 50 minutes to promote cDNA synthesis, and the reaction was terminated by heating up to 85 °C for 5 minutes and then snap cooling at 0 °C for 1 minute. Finally, RNase H (1 μL, 2 U/mL) was added and incubated at 37 °C for 20 minute to remove RNA strands from RNA-cDNA hybrids. Synthesized cDNA was either stored at –20 °C or used immediately for real-time reverse transcriptase polymerase chain reaction (RT-qPCR). To perform RT-qPCR utilizing a Light Cycler 480 (Roche Applied Science), Taq-Man Gene Expression Assays (Applied Biosystems) were used as a primer set specified for 11 types of collagens, 6 types of proteoglycans, and 8 types of glycoproteins (Supplementary Table 1).

The PCRs were performed in a final volume of 20 μL containing 10 μL 2× LightCycler 480 Probes Master (FastStart Taq DNA polymerase, reaction buffer, dNTP mix [with dUTP instead of dTTP], and 6.4 mM MgCl2) (Roche Applied Science), 1 μL TaqMan Gene Expression Assay (Applied Biosystems), 5 μL cDNA as the template, and 4 μL H2O using the following program: 95 °C for 10 minutes, 40 cycles at 95 °C for 10 seconds, and 60 °C for 1 minute, followed by 72 °C for 4 seconds, and a final cooling at 40 °C for 30 seconds. Experiments were performed in triplicate, and average values were calculated for normalized expression levels. During PCR amplification, amplified product amounts were monitored by continuous measurement of fluorescence. Gene expressions were normalized versus glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as follows: the cycle number at which the transcript of each gene was detectable (threshold cycle, Ct) was normalized against the Ct of GAPDH, which is referred to as ΔCt. The $2^{-\Delta\Delta CT}$ method was used to analyze the relative gene expression levels using GAPDH as an endogenous control.

Table 1. Demographic Characteristics of Patients

Variable	Control (n = 3)	RCT (n = 12)	*p*-value
Age (yr)	64.3 ± 3.1	60.1 ± 10.2	0.385*
Sex			0.126†
Male	1 (33)	9 (75)	
Female	2 (67)	3 (25)	
Symptom duration (mo)	-	10.8 ± 16.9	-
Symptom aggravation (mo)	-	3.0 ± 2.3	-

Values are presented as mean ± standard deviation or number (%).
RCT: rotator cuff tear.
*Mann-Whitney test. †Chi-square test.

Statistical Analysis

Data related to the study design are presented as mean ± standard deviation, and gene expression values are presented as mean ± standard deviation. Statistical differences between gene expression values detected in the normal and RCTs groups were analyzed by Mann-Whitney *U*-test, and level of significance was set at $p = 0.05$. For non-parametric multiple comparisons among the 4 tear sizes, statistical differences were analyzed by Mann-Whitney *U*-test with Bonferroni correction in each group. The level of significance was set at $p = 0.008$. Statistical analyses were performed using SPSS version 22.0 (IBM Corp.).

RESULTS

Twelve patients with full-thickness RCTs were included in the experimental group, which had an average age of 60.1 years (range, 45–81 years) and a male to female ratio of 3:1, and the symptom or sign duration was 10.8 months (range, 1–60 months) (Table 1). All patients underwent magnetic

Table 2. Information about the RCT Groups

Variable	Small	Medium	Large	Massive
Tear size (mm)	9.5 ± 0.7	19.7 ± 7.1	39.0 ± 6.0	56.0 ± 5.3
Retraction (mm)	13.5 ± 6.4	16.3 ± 8.6	25.3 ± 15.0	40.0 ± 14.8

Values are presented as mean ± standard deviation.
RCT: rotator cuff tear.

resonance imaging and then were further divided into small, medium, large, and massive size RCT groups according to Cofield's classification (Table 2). The control group included 3 patients who had proximal humerus fractures but no RCT. Their average age was 64.3 years (range, 61–67 years) and a male to female ratio was 1:2. All patients underwent arthroscopic surgery and recovered well after surgery without related complications.

To investigate the difference in ECM gene expression in RCTs in the normal group, we compared 11 collagens and 14 NCPs, which were 6 proteoglycans and 8 glycoproteins (Table 3). In the case of collagens, 9 genes

Table 3. The Expression of 25 ECM Genes in Normal and Rotator Cuff Tear Tendons

Category	Change (vs. normal)	Gene	Relative gene expression		*p*-value
			Normal (n = 3)	RCT (n = 12)	
Collagen	Upregulated	*COL1A1*	1.00 ± 0.02	5.13 ± 1.35	0.843
		COL2A1	1.00 ± 0.08	10.16 ± 3.34	0.307
		COL3A1	1.00 ± 0.01	2.91 ± 0.39	0.045*
		COL5A1	1.00 ± 0.06	6.18 ± 2.21	0.679
		COL6A1	1.00 ± 0.02	9.41 ± 1.63	0.125
		COL9A1	1.00 ± 0.09	6.52 ± 2.37	0.671
		COL10A1	1.00 ± 0.09	2.70 ± 1.10	0.018*
		COL11A1	1.00 ± 0.07	1.78 ± 0.61	0.192
		COL12A1	1.00 ± 0.03	1.49 ± 0.28	0.943
	Downregulated	*COL4A1*	1.00 ± 0.37	0.25 ± 0.10	0.001*
		COL14A1	1.00 ± 0.38	0.28 ± 0.07	0.000*
Proteoglycan	Upregulated	*ACAN*	1.00 ± 0.07	8.92 ± 1.69	0.000*
		BGN	1.00 ± 0.01	4.91 ± 1.85	0.798
		FMOD	1.00 ± 0.02	4.02 ± 1.25	0.192
		VCAN	1.00 ± 0.02	2.05 ± 0.59	0.777
	Downregulated	*DCN*	1.00 ± 0.04	0.18 ± 0.03	0.000*
		LUM	1.00 ± 0.04	0.85 ± 0.12	0.042*
Glycoprotein	Upregulated	*COMP*	1.00 ± 0.04	12.14 ± 4.93	0.053
		FN1	1.00 ± 0.05	3.47 ± 1.80	0.003*
		FBN1	1.00 ± 0.11	7.99 ± 2.07	0.487
		HAPLN1	1.00 ± 0.04	4.64 ± 1.31	0.334
		TNC	1.00 ± 0.05	5.38 ± 1.84	0.005*
	Downregulated	*ELN*	1.00 ± 0.03	0.07 ± 0.04	0.000*
		LAMA2	1.00 ± 0.06	0.11 ± 0.04	0.000*
		THBS1	1.00 ± 0.03	0.44 ± 0.08	0.002*

Values are presented as mean ± standard error.
ECM: extracellular matrix, RCT: rotator cuff tear, *COL*: collagen, *ACAN*: aggrecan, *BGN*: biglycan, *FMOD*: fibromodulin, *VCAN*: versican, *DCN*: decorin, *LUM*: lumican, *COMP*: cartilage oligomeric matrix protein, *FN1*: fibronectin 1, *FBN1*: fibrillin 1, *HAPLN1*: hyaluronan and proteoglycan link protein 1, *TNC*: tenascin C, *ELN*: elastin, *LAMA2*: laminin subunit alpha 2, *THBS1*: thrombospondin 1.
*Statistically significant, $p < 0.05$.

(*COL1A1, COL2A1, COL3A1, COL5A1, COL6A1, COL9A1, COL10A1, COL11A1*, and *COL12A1*) showed a tendency to increase in the RCT patients, and there was a significant increase in COL3A1 (2.91-fold, $p = 0.045$) and COL10A1 (2.70-fold, $p = 0.018$) compared to those in the normal group. In addition, there was a significant tendency to decline in the other 2 genes: COL4A1 (0.25 ± 0.10, $p = 0.001$) and COL14A1 (0.28 ± 0.07, $p < 0.001$). In the case of NCPs, 4 genes (*ACAN, BGN, FMOD*, and *VCAN*) were increased in the RCT patients, and ACAN was significantly increased by 8.92-fold ($p < 0.001$) compared to that in the normal group. *DCN* and *LUM* showed a tendency to decrease in the RCT patients, with a significant decrease of 0.18-fold ($p < 0.001$) and 0.85-fold ($p = 0.042$), respectively. In the case of glycoproteins, the expression of 6 genes (*COMP, FN1, FBN1, HAPLN1*, and *TNC*) increased in the RCT patients, *FN1* and *TNC* increased significantly by 3.47-fold ($p = 0.003$) and 5.38-fold ($p = 0.005$), respec-

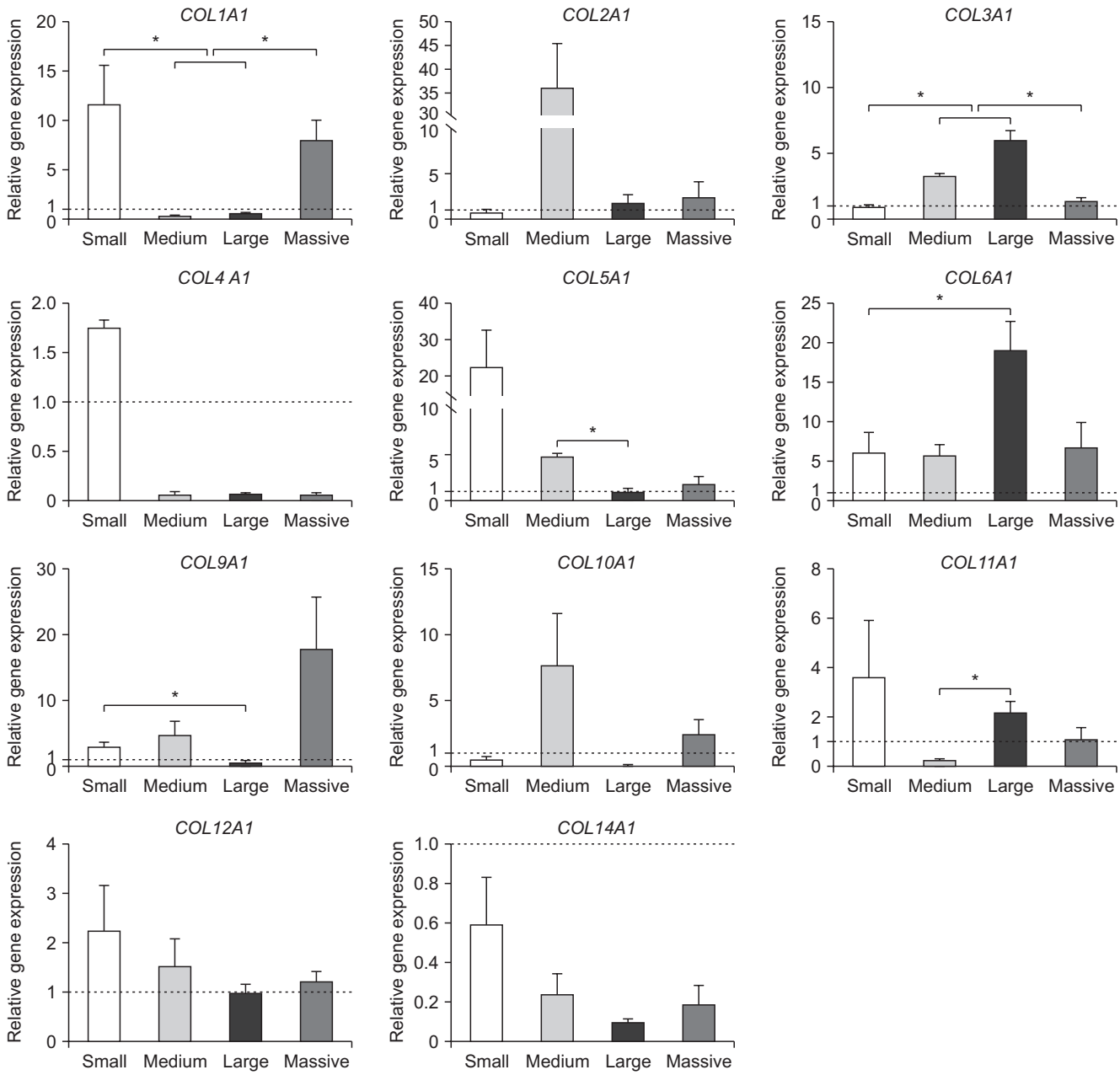

Fig. 1. Comparison of gene expressions of 11 collagens in small, medium, large, and massive size rotator cuff tears. The dashed lines represent gene expression levels of the normal control group and gene expression in each size was normalized relative to the normal group. Values are presented as mean ± standard error. *Statistically significant, $p < 0.05$.

tively, compared to those in the normal group. The genes *ELN, LAMA2*, and *THBS1* were all significantly reduced (0.07-fold, $p < 0.001$; 0.11-fold, $p < 0.001$; and 0.44-fold, $p = 0.002$). Taken together, these results showed that the SSTs in the RCT patients showed a difference in ECM gene expression compared to the normal controls.

To investigate the relationship between changes in ECM gene expression and the size of the RCTs, we analyzed individual ECM gene including collagens and NCPs by dividing the tear size into small, medium, large, and massive (Table 2). For collagens, among the 9 collagens with increased gene expression compared to the normal group (Table 3), the following genes showed the highest expression in small size RCTs and decreased as the size increased: *COL1A1* (11.65-fold), *COL5A1* (22.54-fold), *COL11A1* (3.61-fold), and *COL12A1* (2.24-fold). The *COL1A1* showed a statistical difference when compared to the other sizes (vs. medium and large). In the medium size, the genes showing the highest level of expression and decreasing as the size increases were *COL2A1* (35.7-fold) and *COL10A1* (7.67-fold), but there was no significant difference from other sizes (Fig. 1). The most expressed in large size were *COL3A1* and *COL6A1*, with significant differences between the other sizes (*COL3A1* vs. small and massive; *COL6A1* vs. small). *COL9A1* had the highest level of expression (17.77-fold) in the massive size; however, there was no significant difference when compared to the other sizes. In the 2 collagen genes (*COL4A1* and *COL14A1*) that had decreased gene expression compared to the normal group, higher expression levels were observed in small sizes (1.76-fold and 0.59-fold). Among the 11 collagens tested, the gene expression trend varied with the change in size.

For proteoglycans, 3 out of the 4 genes (*ACAN, BGN*, and *FMOD*) showed the highest levels of expression in small size RCTs (13.3-fold, 14.27-fold, and 8.74-fold, respectively) compared to those in the normal group (Fig. 2). There was no significant difference detected when compared to the other sizes. The VCAN had the highest expression level in medium size RCTs (5.15-fold), followed by the small size RCTs (2.14-fold). There were significant differences between the expression levels of the other sizes (small vs. large and massive; medium vs. large). The gene DCN had the highest expression level in small size RCTs (0.29-fold) among the 2 genes with reduced expression. For glycoproteins, all the 5 genes with elevated expression (*COMP, FBN1, FN1, HAPLN1,* and *TNC*) were the highest in small size RCTs (34.12-fold, 20.96-fold, 12.63-fold, 14.16-fold, and 15.6-fold), and the expression levels of *FBN1* and *TNC* genes were statistically different from those of the other sizes (Fig. 3). The expression levels of *THBS1* (0.08-fold), *ELN* (0.33-fold), and *LAMA2* (0.24-fold) genes were decreased in all sizes compared to those in the normal group. In the NCPs, almost all the genes

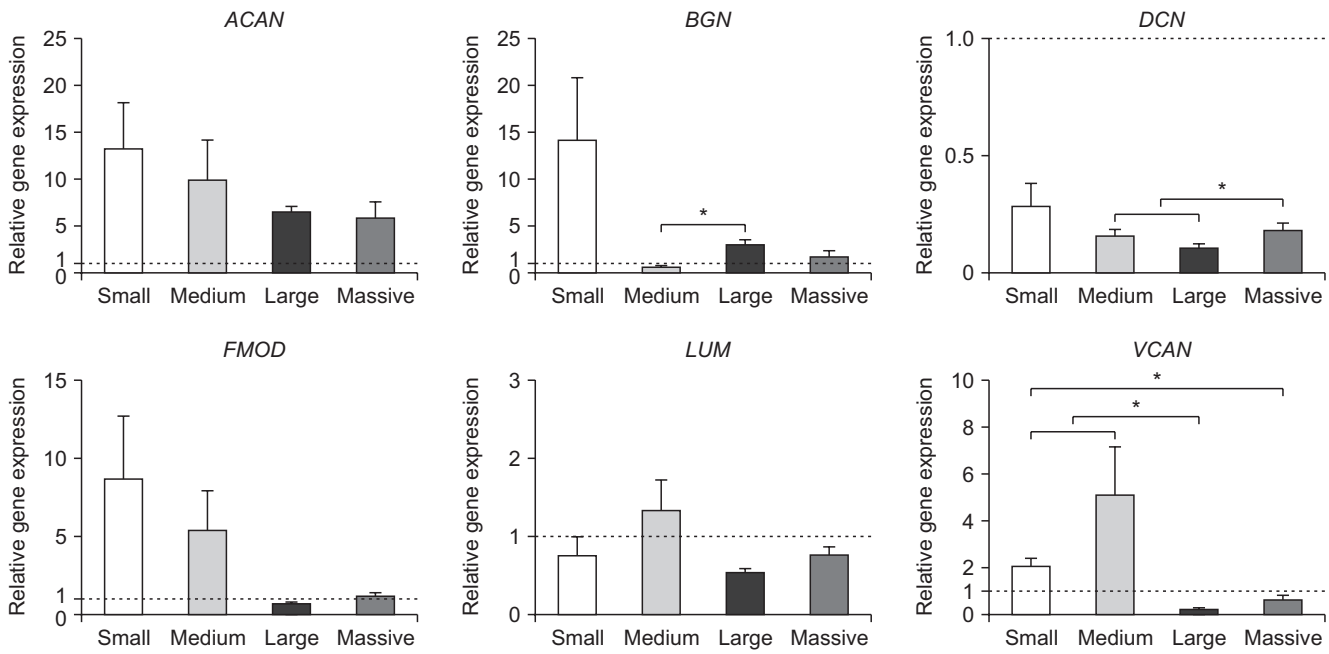

Fig. 2. Comparison of gene expressions of 6 proteoglycans in small, medium, large, and massive size rotator cuff tears. The dashed lines represent gene expression levels of the normal control group and gene expression in each size was normalized relative to the normal group. Values are presented as mean ± standard error. *Statistically significant, $p < 0.05$.

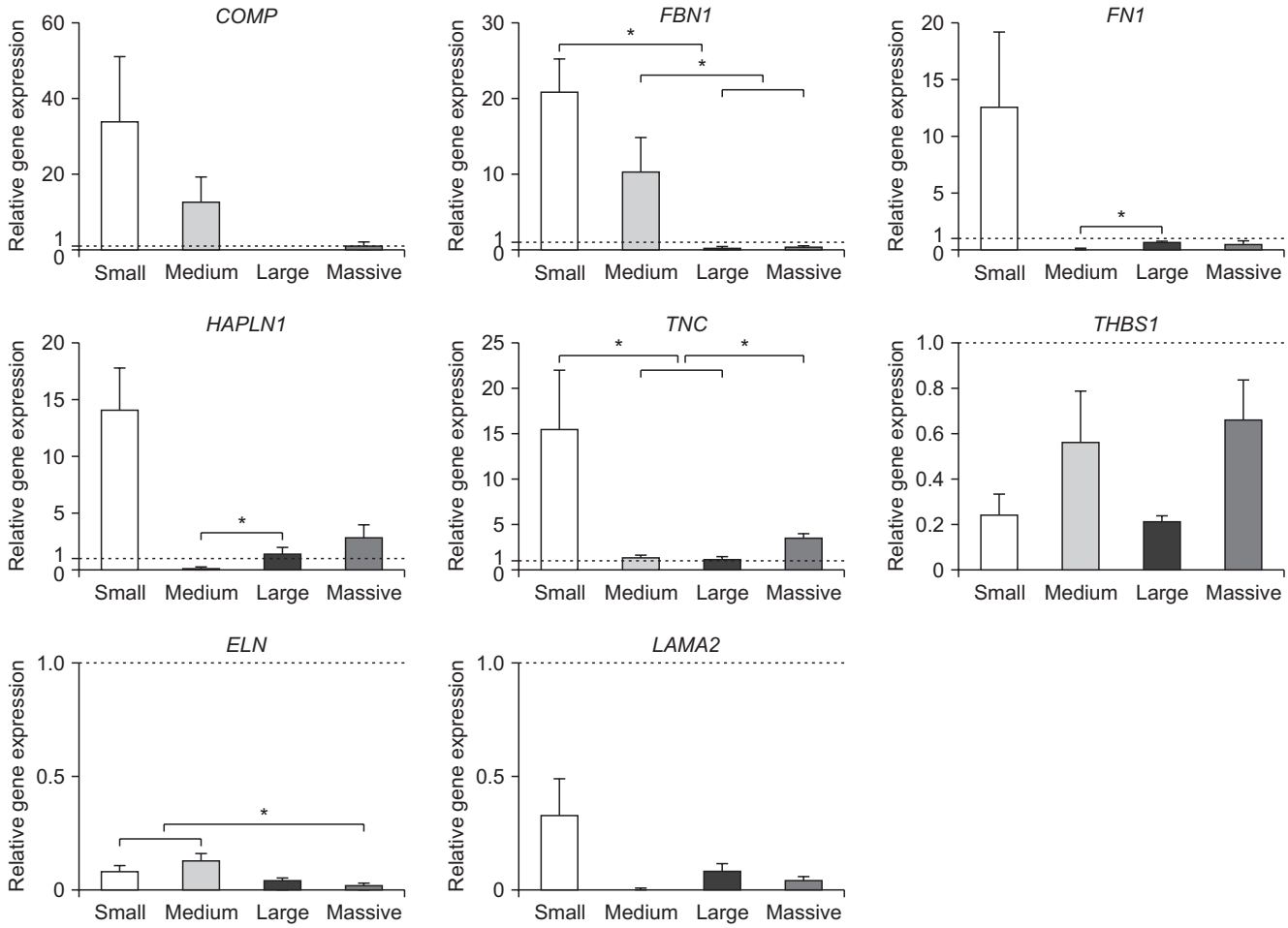

Fig. 3. Comparison of gene expressions of 8 glycoproteins in small, medium, large, and massive size rotator cuff tears. The dashed lines represent gene expression levels of the normal control group and gene expression in each size was normalized relative to the normal group. Values are presented as mean ± standard error. *Statistically significant, $p < 0.05$.

with increased expression levels had the highest level in small size RCTs, and the gene expression decreased with the increase of size.

DISCUSSION

The most important findings of this study are as follows: (1) SST of RCT patients showed significant difference in ECM gene expression level, including 4 collagens and 8 NCPs, compared to normal tendon; (2) In small RCTs, most of the NCPs, 3 out of 6 proteoglycans and 5 out of 8 glycoproteins, showed an increased gene expression level from 5.15- to 34.12-fold, followed by a decreased level in the other sizes. However, collagens exhibited various gene expression patterns across all sizes. These results suggest that the change in NCP gene expression is more susceptible than that of collagens after small sizes in RCT.

In this study, we analyzed the 25 ECM genes from SSTs of RCT patients in comparison to normal tendons (Table 3). Among collagens, *COL3A1* and *COL10A1* exhibited a significant increase of 2.91- and 2.70-fold, respectively, while *COL4A1* and *COL14A1* showed significant decreases of 0.25- and 0.28-fold, respectively. Among the NCPs, *ACAN, FN1*, and *TNC* demonstrated significant increases of 8.92-, 3.47-, 5.38-fold, respectively, whereas *DCN, LUM, ELN*, and *LAMA2* exhibited significant decreases of 0.18-, 0.85-, 0.07-, and 0.11-fold, respectively. Including these 7 ECM genes, the tendency of change for all the ECM genes analyzed herein was consistent with previous results, which analyzed several ECM components using RCT tissues. The observed differences in statistical significance compared to previous studies may arise from the use of different controls and sample sizes. These findings suggest that ECM gene expression is differentially regulated in response to an RCT indicating that ECM gene regulation is a consequence of an RCT, rather than a cause.

Although the factors contributing to the progression of an RCT are not fully understood, most studies demonstrate that it is from tendinopathy to a partial-thickness tear, and then a full-thickness tear.[20] This progression is associated with an increase in tear size and a deterioration of tendon quality. Thus, tear size is known as one of the critical risk factors closely related to poor outcomes of repair for large to massive than small RCTs.[21,22] In practice, Galatz et al.[3] reported 94.4% retear rate was observed after completing arthroscopic repair of medium to massive (> 2 cm) RCTs. Recently, Chaudhury et al.[16] categorized SST tear sizes into 2 groups, small and large, and analyzed overall gene profiles differentially expressed in normal, small, and large tears. They found downregulation of type IV, XII, and XIV collagen genes in small tears compared to large tears, and upregulation of aggrecan in large tendon tears relative to normal controls. These findings are consistent with the results of type IV, XII, and XIV collagens and aggrecan herein when we would divide SST tear size into 2 groups (Figs. 1 and 2). For a more detailed analysis, we assessed ECM gene expression change across a series of tear sizes, with a hypothesis that ECM gene expression varies with the extent of tear size. We categorized RCT sizes into 4 groups: small, medium, large, and massive and identified a characteristic pattern where most of the NCPs genes, 11 out of 14 genes of proteoglycans and glycoproteins we tested, showed an increase from 2.14- to 34.12-fold in small tears compared to the normal tendon, followed by a significant decrease in medium, large, and massive tears (Fig. 2 and 3). Moreover, the expression levels of 10 of the 14 NCP genes were lower than those in the normal tendon in cases of either large or massive tears. In contrast, the collagen expression pattern varied across different sizes of tears. These results suggest that differential expression of ECM genes, especially NCP genes, in large to massive RCTs may be a significant factor to consider in the treatment of RCTs.

Proteoglycans are pivotal in regulating the assembly of the major structural component of the tendon, type I collagen, and mediate the viscoelastic mechanical properties of tendons, including tension, shear, and compression. They contribute to compressive stiffness through their glycosaminoglycan contents, which are negatively charged hydrophilic molecules capable of retaining water 50 times their weight.[10,23] Thus, impaired expression of proteoglycans or glycoproteins leads to weak tendon formation with abnormal collagen fibrils and a reduction in stiffness. For example, biglycan/fibromodulin double knockout mice exhibit weakened tendons with abnormal collagen morphology, a 56.6% reduction in stiffness, and ectopic tendon ossification as a compensatory mechanism for diminished stiffness.[24] Similarly, other NCPs, glycoproteins are essential for tendon maturation and the physiology of tendon-resident cells.[17,25,26] These results suggest that the expression of NCPs is a crucial factor for tendon quality. Several studies have shown that treatment with bioactive materials significantly enhances tenocyte proliferation and upregulates the gene expression of both collagens and NCPs in tenocytes.[27,28] Furthermore, direct administration of NCPs such as tenascin C protein and fibromodulin gene has been demonstrated to facilitate tendon healing in vivo.[29,30] Taken together, these results indicate the importance of NCPs as potential targets for enhancing tendon repair and regeneration.

This study has several limitations. First, we analyzed collagens and NCPs excluding other ECM regulatory proteins such as matrix metalloproteinases, tissue inhibitors of metalloproteinases, and a disintegrin and metalloproteinase with thrombospondin motifs. To study the regulation of these molecule would provide deeper insights into tendon remodeling and its relation to wound healing for RCT treatment. Second, this study focused on the gene expression level of ECM components, without validating these changes at the protein level. Further studies confirming these alterations in both collagen and NCPs at the protein level would be necessary to demonstrate actual physiological changes. Third, a large sample size may be required to enable more detailed analysis and ensure the robustness of the findings.

We confirmed that most of the detected ECM gene expression changes were related to the size of the full-thickness RCTs. Especially in NCPs, gene expression was increased in small-size tears, and gene expression levels were significantly reduced when the size increased. These results may indicate one of the reasons for the poor healing of larger than small size tears and provide a greater possibility of better healing for early intervention.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGEMENTS

This research was supported by a grant (HI20C0386) of the Korea Health Industry Development Institute (KHIDI), a grant (No. 2022R1A2C2092854) of the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT), a grant (S3283968) of the Technology Development Program funded by the Ministry

of SMEs and Startups (MSS, Korea), a grant (22C0608L1) of the Korean Fund for Regenerative Medicine funded by Ministry of Science and ICT, and Ministry of Health and Welfare, and a grant (04-2024-0010) of the focused clinical research grant-in-aid from Seoul Metropolitan Government Seoul National University (SMG-SNU) Boramae Medical Center.

ORCID

Jian Jiang https://orcid.org/0009-0006-7198-3966
Kwi-Hoon Jang https://orcid.org/0000-0002-3177-2965
Sung Yong Ahn https://orcid.org/0000-0002-6029-1853
Chris Hyunchul Jo https://orcid.org/0000-0002-6161-5442

SUPPLEMENTARY MATERIAL

Supplementary material is available in the electronic version of this paper at the CiOS website, www.ecios.org.

REFERENCES

1. Garibaldi R, Altomare D, Sconza C, et al. Conservative management vs. surgical repair in degenerative rotator cuff tears: a systematic review and meta-analysis. Eur Rev Med Pharmacol Sci. 2021;25(2):609-19.
2. Kukkonen J, Kauko T, Virolainen P, Aarimaa V. The effect of tear size on the treatment outcome of operatively treated rotator cuff tears. Knee Surg Sports Traumatol Arthrosc. 2015;23(2):567-72.
3. Galatz LM, Ball CM, Teefey SA, Middleton WD, Yamaguchi K. The outcome and repair integrity of completely arthroscopically repaired large and massive rotator cuff tears. J Bone Joint Surg Am. 2004;86(2):219-24.
4. DeOrio JK, Cofield RH. Results of a second attempt at surgical repair of a failed initial rotator-cuff repair. J Bone Joint Surg Am. 1984;66(4):563-7.
5. Matthews TJ, Hand GC, Rees JL, Athanasou NA, Carr AJ. Pathology of the torn rotator cuff tendon. Reduction in potential for repair as tear size increases. J Bone Joint Surg Br. 2006;88(4):489-95.
6. Moosmayer S, Tariq R, Stiris M, Smith HJ. The natural history of asymptomatic rotator cuff tears: a three-year follow-up of fifty cases. J Bone Joint Surg Am. 2013;95(14):1249-55.
7. Park JG, Cho NS, Song JH, Baek JH, Jeong HY, Rhee YG. Rotator cuff repair in patients over 75 years of age: clinical outcome and repair integrity. Clin Orthop Surg. 2016;8(4):420-7.
8. Arnoczky SP, Lavagnino M, Egerbacher M. The mechanobiological aetiopathogenesis of tendinopathy: is it the over-stimulation or the under-stimulation of tendon cells? Int J Exp Pathol. 2007;88(4):217-26.
9. Lee GW, Kim JY, Lee HW, Yoon JH, Noh KC. Clinical and anatomical outcomes of arthroscopic repair of large rotator cuff tears with allograft patch augmentation: a prospective, single-blinded, randomized controlled trial with a long-term follow-up. Clin Orthop Surg. 2022;14(2):263-71.
10. Eisner LE, Rosario R, Andarawis-Puri N, Arruda EM. The role of the non-collagenous extracellular matrix in tendon and ligament mechanical behavior: a review. J Biomech Eng. 2022;144(5):050801.
11. Screen HR, Berk DE, Kadler KE, Ramirez F, Young MF. Tendon functional extracellular matrix. J Orthop Res. 2015;33(6):793-9.
12. Watt FM, Huck WT. Role of the extracellular matrix in regulating stem cell fate. Nat Rev Mol Cell Biol. 2013;14(8):467-73.
13. Santoro Belangero P, Antonio Figueiredo E, Cohen C, et al. Changes in the expression of matrix extracellular genes and TGFB family members in rotator cuff tears. J Orthop Res. 2018;36(9):2542-53.
14. Eager JM, Warrender WJ, Deusenbery CB, et al. Distinct gene expression profile in patients with poor postoperative outcomes after rotator cuff repair: a case-control study. Am J Sports Med. 2021;49(10):2760-70.
15. Tashjian RZ, Lock I, Granger EK, et al. Gene expression in torn rotator cuff tendons determined by RNA sequencing. Orthop J Sports Med. 2020;8(6):2325967120927480.
16. Chaudhury S, Xia Z, Thakkar D, Hakimi O, Carr AJ. Gene expression profiles of changes underlying different-sized human rotator cuff tendon tears. J Shoulder Elbow Surg. 2016;25(10):1561-70.
17. Taye N, Karoulias SZ, Hubmacher D. The "other" 15-40%: the role of non-collagenous extracellular matrix proteins and minor collagens in tendon. J Orthop Res. 2020;38(1):23-35.
18. Jo CH, Kim JE, Yoon KS, et al. Does platelet-rich plasma accelerate recovery after rotator cuff repair? A prospective cohort study. Am J Sports Med. 2011;39(10):2082-90.
19. Jo CH, Kim OS, Park EY, et al. Fetal mesenchymal stem cells derived from human umbilical cord sustain primitive characteristics during extensive expansion. Cell Tissue Res. 2008;334(3):423-33.

20. Factor D, Dale B. Current concepts of rotator cuff tendinopathy. Int J Sports Phys Ther. 2014;9(2):274-88.

21. Longo UG, Carnevale A, Piergentili I, et al. Retear rates after rotator cuff surgery: a systematic review and meta-analysis. BMC Musculoskelet Disord. 2021;22(1):749.

22. Wang PW, Jo CH. Prognostic factors affecting structural integrity after arthroscopic rotator cuff repair: a clinical and histological study. Clin Shoulder Elb. 2023;26(1):10-9.

23. Yoon JH, Halper J. Tendon proteoglycans: biochemistry and function. J Musculoskelet Neuronal Interact. 2005;5(1):22-34.

24. Ameye L, Aria D, Jepsen K, Oldberg A, Xu T, Young MF. Abnormal collagen fibrils in tendons of biglycan/fibromodulin-deficient mice lead to gait impairment, ectopic ossification, and osteoarthritis. FASEB J. 2002;16(7):673-80.

25. Singh P, Carraher C, Schwarzbauer JE. Assembly of fibronectin extracellular matrix. Annu Rev Cell Dev Biol. 2010; 26:397-419.

26. Docheva D, Hunziker EB, Fassler R, Brandau O. Tenomodulin is necessary for tenocyte proliferation and tendon maturation. Mol Cell Biol. 2005;25(2):699-705.

27. Jo CH, Kim JE, Yoon KS, Shin S. Platelet-rich plasma stimulates cell proliferation and enhances matrix gene expression and synthesis in tenocytes from human rotator cuff tendons with degenerative tears. Am J Sports Med. 2012;40(5):1035-45.

28. Qian H, Meng J, Yuan T, et al. Gene expression in synovium of rotator cuff tear patients determined by RNA sequencing. Biochem Genet. 2024;62(1):452-67.

29. Xu K, Shao Y, Xia Y, et al. Tenascin-C regulates migration of SOX10 tendon stem cells via integrin-α9 for promoting patellar tendon remodeling. Biofactors. 2021;47(5):768-77.

30. Delalande A, Gosselin MP, Suwalski A, et al. Enhanced Achilles tendon healing by fibromodulin gene transfer. Nanomedicine. 2015;11(7):1735-44.